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WHAT IS CLAIMED IS:

A Granulocyte Colony Stimulating Factor peptide comprising the moiety: 1 1.

$$\begin{array}{c} OH \\ O \\ G-HN \end{array} \qquad \begin{array}{c} OH \\ O \\ O \\ \end{array}$$

wherein 3

D is a member selected from -OH and R¹-L-HN-; 4

G is a member selected from R^1 -L- and -C(O)(C_1 - C_6)alkyl; 5

R¹ is a moiety comprising a member selected a moiety comprising a straight-6 7

chain or branched poly(ethylene glycol) residue; and

L is a linker which is a member selected from a bond, substituted or 8 unsubstituted alkyl and substituted or unsubstituted heteroalkyl, 9

such that when D is OH, G is R^1 -L-, and when G is $-C(O)(C_1-C_6)$ alkyl, D is 10

R1-L-NH-. 11

> The peptide according to claim 1, wherein L-R¹ has the formula: 2.

2

3 wherein

4 a is an integer from 0 to 20.

The peptide according to claim 1, wherein R¹ has a structure that is a member 3. 1

2 selected from:

4 wherein

e and f are integers independently selected from 1 to 2500; and

6 q is an integer from 0 to 20.

1 4. The peptide according to claim 1, wherein R¹ has a structure that is a member

2 selected from:

3

4 wherein

e, f and f' are integers independently selected from 1 to 2500; and

q and q' are integers independently selected from 1 to 20.

and

- 1 5. The peptide according to claim 1, wherein R¹ has a structure that is a member
- 2 selected from:

4 wherein

e, f and f' are integers independently selected from 1 to 2500; and

q, q' and q"are integers independently selected from 1 to 20.

1 6. The peptide according to claim 1, wherein R¹ has a structure that is a member

2 selected from:

3

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$$\xi$$
—C(O)CH2CH2(OCH2CH2)eOCH3 ; and

4 wherein

5 e and f are integers independently selected from 1 to 2500.

1 7. The G-CSF peptide according to claim 1, wherein said moiety has the

2 formula:

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1 8. The G-CSF peptide according to claim 1, wherein said moiety has the

2 formula:

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1 9. The G-CSF peptide according to claim 1, wherein said moiety has the

2 formula:

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4 wherein

5 AA is an amino acid residue of said peptide.

- 1 10. The G-CSF peptide according to claim 9, wherein said amino acid residue is a
- 2 member selected from serine or threonine.
- 1 11. The G-CSF peptide according to claim 1, wherein said peptide has the amino
- 2 acid sequence of SEQ. ID. NO:1.
- 1 12. The G-CSF peptide according to claim 11, wherein said amino acid residue is
- 2 threonine at position 133 of SEQ. ID. NO:1.
- 1 13. The peptide according to claim 1, wherein said peptide has an amino acid
- 2 sequence selected from SEQ. ID. NO:1 and SEQ ID NO:2.
- 1 14. The G-CSF peptide according to claim 1, wherein said moiety has the
- 2 formula:

$$\xi = AA - \left(\begin{array}{c} (\operatorname{Fuc})_{i} \\ -\operatorname{GlcNAc-GlcNAc-Man} \\ -\operatorname{GlcNAc-GlcNAc-Man} \\ -\operatorname{GlcNAc-GlcNAc-Man} \\ -\operatorname{GlcNAc-GlcNAc-Man} \\ -\operatorname{GlcNAc-GlcNAc-Man} \\ -\operatorname{GlcNAc-GlcNAc-Man} \\ -\operatorname{GlcNAc-GlcNAc-GlcNAc-Man} \\ -\operatorname{GlcNAc-GlcNAc-Man} \\ -\operatorname{GlcNAc-Man} \\ -\operatorname{Glc$$

3 4 wherein a, b, c, d, i, r, s, t, and u are integers independently selected from 0 and 1; 5 6 e, f, g, and h are members independently selected from the integers from 0 to 7 8 9 j, k, l, and m are members independently selected from the integers from 0 and 100; 10 v, w, x, and y are independently selected from 0 and 1, and least one of v, w, x 11 and y is 1; 12 13 AA is an amino acid residue of said G-CSF peptide; Sia-(R) has the formula: 14 NH-G 15 16 wherein D is a member selected from -OH and R¹-L-HN-; 17 G is a member selected from R^1 -L- and -C(O)(C₁-C₆)alkyl; 18 R¹ is a moiety comprising a member selected a straight-chain or 19 branched poly(ethylene glycol) residue; and 20 L is a linker which is a member selected from a bond, substituted or 21 22 unsubstituted alkyl and substituted or unsubstituted heteroalkyl, such that when D is OH, G is R^1 -L-, and when G is $-C(O)(C_1-C_6)$ alkyl, 23 D is R¹-L-NH-. 24

1 15. The peptide according to claim 14, wherein said amino acid residue is an

- 2 asparagine residue.
- 1 16. The peptide according to claim 1, wherein said peptide is a bioactive
- 2 Granulocyte Colony Stimulating Factor peptide.
- 1 17. A method of making a G-CSF peptide conjugate comprising the moiety:

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3 wherein

D is a member selected from -OH and R¹-L-HN-;

G is a member selected from R^1 -L- and -C(O)(C_1 - C_6)alkyl;

R¹ is a moiety comprising a member selected a straight-chain or branched poly(ethylene glycol) residue; and

8 L is a linker which is a member selected from a bond, substituted or

9 unsubstituted alkyl and substituted or unsubstituted heteroalkyl,

such that when D is OH, G is R¹-L-, and when G is -C(O)(C₁-C₆)alkyl, D is

11 R¹-L-NH-,

said method comprising:

(a) contacting a substrate G-CSF peptide with a PEG-sialic acid donor moiety having the formula:

15 16

17

12

13

14

and an enzyme that transfers said PEG-sialic acid onto an amino acid or glycosyl residue of said G-CSF peptide, under conditions

appropriate for the transfer.

1 18. The method according to claim 17, wherein L-R¹ has the formula:

23 wherein

- 4 a is an integer from 0 to 20.
- 1 19. The method according to claim 17, wherein R¹ has a structure that is a
- 2 member selected from:

4 wherein

3

- 5 e and f are integers independently selected from 1 to 2500; and
- 6 q is an integer from 0 to 20.
- 1 20. The method according to claim 17, wherein R¹ has a structure that is a
- 2 member selected from:

4 wherein

3

e, f and f' are integers independently selected from 1 to 2500; and

6 q and q' are integers independently selected from 1 to 20.

1 21. The method according to claim 17, wherein R¹ has a structure that is a

2 member selected from:

and

4 wherein

3

e, f and f' are integers independently selected from 1 to 2500; and

q, q' and q"are integers independently selected from 1 to 20.

- 1 22. The method according to claim 17, wherein R¹ has a structure that is a
- 2 member selected from:

$$\label{eq:coch2} \begin{split} & \frac{1}{2} - \text{C(O)CH}_2\text{CH}_2\text{(OCH}_2\text{CH}_2\text{)}_e\text{OCH}_3 \;\; ; \text{ and} \end{split}$$

$$\xi$$
—C(O)OCH₂CH₂(OCH₂CH₂)_fOCH₃

4 wherein

3

5 e and f are integers independently selected from 1 to 2500.

- 1 23. The method of claim 17, further comprising, prior to step (a):
- 2 (b) expressing said substrate Granulocyte Colony Stimulating Factor
- 3 peptide in a suitable host.
- 1 24. The method of claim 17, wherein said host is selected from an insect cell and a
- 2 mammalian cell.
- 1 25. A method of stimulating inflammatory leukocyte production in a mammal,
- 2 said method comprising administering to said mammal a peptide according to claim1.

1 26. A method of treating infection in a subject in need thereof, said method

- 2 comprising the step of administering to the subject an amount of a peptide according
- 3 to claim 1, effective to ameliorate said condition in said subject.
- 1 27. A pharmaceutical formulation comprising the Granulocyte Colony Stimulating
- 2 Factor peptide according to claim 1, and a pharmaceutically acceptable carrier.
- 1 28. A method of refolding an insoluble recombinant granulocyte colony
- 2 stimulating factor (GCSF) protein, the method comprising the steps of:
- 3 (a) solubilizing the GCSF protein; and
- 4 (b) contacting the soluble GCSF protein with a buffer comprising a
- 5 redox couple to refold the GCSF protein, wherein the refolded GCSF protein is
- 6 biologically active.

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